EXTRACTION OF PROTEIN FROM THE SOY BEAN

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Extraction of Protein from Soy Bean

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OBJECT.

The industrial extraction of proteins from the soy bean seems to be a thoroughly commercial proposition. There are large enough crops of this bean grown in the United States, and in European and Asiatic countries, to supply an almost unlimited demand.

The extracted proteins would be chiefly used as a substitute for casein. Casein is itself a protein contained in the soy bean. This bean also contains other proteins of a very similar character, which can be used for exactly the same purposes as casein. The proteins found in the soy bean have a colloidal character similar to that of casein, rendering them especially useful as an adhesive. The paper industry offers an ever widening field for the use of casein. Cardboard, water flasks, buckets, bags and wrapping paper can be made water proof by impregnating them with a casein solution and subsequently subjecting them to formaldahyde vapors. Paper slates and drawing papers may be

made erasable by coating them with a casein solution to which finely divided magnesium or lime has been added. The sizing and enameling of paper in this manner has reached remarkable proportions.

Casein can also be used in the manufacture of paints, plastic masses, dyeing and printing textile fabrics, food, medicines, soap, photographic plates, roofing pulp, and many other articles.

In addition to extracting the proteins from the soy bean other parts of the bean must also be utilized, so that the proteins can be marketed at a lower price than the casein for which they are a substitute.

The author wishes to acknowledge his indebtedness to Professor McCormack for his aid in this investigation.

PART ONE

THE SOY BEAN

EXTRACTION OF PROTEINS FROM THE SOY BEAN.

THE SOY BEAN.

The soy bean, also called the soya bean, and in North Carolina the stock pea, is an annual leguminous plant, a native of southeastern Asia. It has been cultivated in China and Japan for more than 5000 years and in extent of uses and value is the most important legume grown in these countries. Within the past few years the soy bean has become a crop of special importance in the world's commerce, and large shipments of beans, oil and meal have been made from Manchuria to America and European countries. The soy bean, which is very rich in protein, is largely utilized by Asiatic people for food, a great variety of products being prepared from it. The bean also contains a valuable vegetable oil, it is therefore extensively employed in the production of oil and cake.

The soy bean was introduced into the United States as early as 1804, but it is only in the last decade that it has become a crop of

much importance. At the present time it is most largely grown for forage. In many sections, expecially southward and in some parts of the corn belt, a very profitable industry has developed from the growing of the seed. During the past few years the acreage has increased to a very considerable extent. A large yield of seed, the excellent quality of the forage, the ease of growing and harvesting the crop, its freedom from insect enemies and plant diseases, and the possibilities of the seed for the production of oil and meal and as a food all tend to give this crop a high potential importance and assure its greater agricultural development in America.

The soy bean was first introduced into Europe about 1790 and was grown for a large number of years without attracting any attention as a plant of much economic importance. In 1875 Professor Haberlandt, of Vienna, began an extensive series of experiments with this crop and strongly urged its use as a food for man and animals. Although interest was increased in its

cultivation during the experiments, the soy bean failed to become of any great importance in Europe. At the present time it is cultivated only to a limited extent in Germany, southern Russia, France and Italy.

Attempts have been made at various times to introduce the soy bean and its products into European markets in competition with other oil seeds. Owing to the inferior quality of the beans and cake received, these efforts were generally unsuccessful. About 1908, the first large trial shipment of beans was made to England. As these were received in much better condition than those of previous shipments, the results obtained were so satisfactory that 1,170,000 tons were shipped in the next three years.

For the development and growth of the soy beans, there should be at least one hundred and twenty five good growing days in the spring, summer and early fall; and, for the later maturing varieties, a little more time than this is

necessary. Outside the more elevated portions of the South, the lack of a sufficient amount of growing weather is not a determining factor. Soy beans may be grown under practically the same conditions as are favorable for the growth of corn. Although they will grow on a diversity of soil, they, like corn, are especially fond of a fine sandy loam or clay loam soil. For the best growth they should have, as is the case with corn, ample moisture in the soil, without the soil containing an excess. When once the crop gets well started and the weather is warm, soy beans grow fairly rapidly, and will make a tremendous amount of growth, if all the conditions are favorable. In North Carolina, where this crop is put in properly on well prepared land and given the necessary cultivation afterwards, it will cost from eight to ten dollars per acre, including the fertilizer, but not the rental of the land, to produce the crop.

The soy bean is an annual two to four feet

high. It has branching hairy stems, with trifoliate, hairy leaves. The flowers are inconspicuous of a pale yellow or violet color, and the fruit is a broad two to five seeded pod, covered like the rest of the plant with stiff reddish hairs. The seeds vary in color from white and yellow, to green, brown and black.

In view of the increasing interest in the soy bean for the production of oil, the percentage of oil is second to yield for seed production alone where the crop is likely to become of importance as an oil seed. At present there are about twenty varieties of the soy bean handled by growers and seedmen in this country. During the past ten years more than 800 lots of seeds for testing, with a view to their introduction into this country, have been received by the United States Department of Agriculture from China, Manchuria, Japan and India. The more important commercial varieties and improved sorts are: 1-Barechet; 2-Biloxi; 3-Black Eyebrow; 4-Chiquita; 5-Early Brown; 6-Elton; 7-Guelph;

8-Haberlandt; 9-Hahto; 10-Hollybrook; 11-Ito San; 12-Lexington; 13-Mammoth; 14-Manchu; 15-Medium Yellow; 16-Mikado; 17-Peking; 18-Shangai; 19-Tokyo; 20-Yokotenn; 21-Virginia; 22-Wilson-Five. The Mammoth is the standard commercial late variety, more extensively grown at the present time than any other.

The yield of soy bean per acre varies with the variety planted, the character of the soil, methods of cultivation, climatic conditions and other productive factors. The yield varies from 1200 to 2000 pounds of shelled seed and 4000 to 5000 of hay per acre. The cost varies from $1.25 to $1.75 per bushel for seeds.

The introduction of the soy bean into the Western World for oil purposes has not made any changes necessary in the equipment in the modern oil mills. The methods used in the extraction of oil are similar to those employed with other seeds, such as cottonseed and linseed. In Manchuria the manufacture of oil and oil cake is not confined to large centers, as a very small

center of bean production has its native mill. The method adopted in these mills is decidedly primitive. The beans are soaked in water over night, then crushed and boiled with a little water, so as to burst the oil cells. They are spread out on iron frames and pressure applied by means of wedges driven in between the cross-beams and beams placed on top of the frames. During the last few years large bean mills equipped with modern machinery have been erected.

A solvent process of extraction, involving the use of benzine, has recently come into use in several English mills, and three such mills are now operated in Manchuria and Japan. The seeds are very finely crushed and then treated directed by the fat solvent. By this process, nearly all the oil is extracted, the meal containing about 1.5 per cent of the oil, and 43 to 45 per cent of protein. It is contended that by the solvent process more oil of a better quality is extracted from the beans. A solvent

process mill erected in Manchuria has a maximum capacity of 80 tons of beans every 24 hours.

In the United States the methods of oil extraction by the oil mills involve only the hydraulic press and the expeller. Either a plate or a cup hydraulic press may be used. When a plate hydraulic press is used the meal is crushed and cooked with water and then put between canvas sheets, the oil being expressed by direct pressure. From 3000 to 3500 pounds per square inch are applied for two hours. Analysis of cake produced by this method show about 9 per cent of oil and 5 per cent by the expeller method.

Soy bean oil is a drying oil and has a rich orange color. The expressed oil, if prepared from thoroughly sound beans, is practically neutral and can find immediate employment for soap making. The rapid introduction of the oil into commerce was due to its soap making qualities, and it stands between linseed oil and cottonseed oil in its general and specific properties

in this respect.

It can be used as a complete substitute for linseed oil in soft soap, but to only a small extent for cottonseed oil in the manufacture of hard soap.

Among the economic values of this bean can be mentioned the following: a human food, concentrated feed for animals, forage for live stock, valuable fertilizer, oil for illuminating purposes, a substitute for butter, oil for the manufacture of soap, the production of glycerine, and for lubricating purposes. The refined oil is used as a substitute for olive oil. Soy bean milk and soy bean cheese are extensively used as an article of diet in the Orient. This cheese is made as follows: one quart of beans is soaked over night in water, then ground and boiled with water enough to make three quarts. This is strained, magnesium chloride being added to precipitate the solution, which is hung in a fine mesh cloth -- and cheese is obtained. Varnishes made from soy bean oil are extensively used,

though when subjected to exposure they do not seem to wear as well as those made from linseed oil. For interior painting purposes, however, these varnishes are equal in every respect to those made from linseed oil. Linseed oil sells on the average at 75 cents per gallon while soy bean oil sells at 55 cents a gallon. During the years from 1911 to 1915 the importation of soy bean oil was as follows:

	Pounds	Dollars
1911	41,105,920	2,555,707.00
1912	28,019,560	1,578,968.00
1913	12,440,406	635,882.00
1914	16,363,645	830,870.00
1915	19,210,028	901,643.00

The soy bean industry has now gained such an importance in Europe that the various countries have been conducting extensive investigations in their African colonies for the production of seed in competition with the Manchuria beans. When soy beans were first imported from Manchuria the price was about $24.00 per ton on the European market, but the competition of the European coun-

tries for the raw product brought the price quickly up to $45.00 per ton, and during the last three years quotations on the different markets average about $40.00 per ton. At these prices it was found that the African colonies were in a favorable position to compete with the bean growers in Manchuria.

Morever, it is evident that the farmer in America is able to compete on the European and home markets both with the Manchurian and African beans at prices prevailing during the last three or four years. Although the selling price f.o.b. Manchurian ports ranges from $30.00 to $35.00 per ton, the transportation makes the price approximately $40.00 at American and European ports.

The soy bean is already a product of high value in American agriculture and seems destined to be of far greater importance, especially in the cotton belt, not only as a cash crop, but as an aid in maintaining the fertility of the soil. With a mutual understanding of the possibilities of the soy bean and its products, the industry

should be a most important one in conjunction with the cotton-oil industry.

An average analysis of the soy bean is as follows:

Oil	18%
Moisture	10%
Proteins	40%
Hydrocarbons	22%
Ash	5%
Fiber	5%

PART TWO

THEORY OF THE PROTEINS

EXTRACTION OF PROTEINS FROM THE SOY BEAN.

THEORY OF THE PROTEINS.

Proteins are found in living parts of all plants. They occur in the dissolved state in the circulating fluids and in the solutions of cell vacuoles, that is in the cell sap. In the semi-dissolved state they occur in the protoplasm, and in the undissolved state as reserve protein in the cells of seeds, tubers, bulbs, buds and roots.

Little that is definite is known concerning the chemical properties of any of the plant proteins except those of the seed, for the proteins occuring in the physiologically active cells and fluids of plants have been but little studied, owing to the relatively small quantities in which they occur and the difficulty of separating them from each other. The proteins of the seed have been the subject of extensive investigation, and we now know much concerning the chemical and physical properties of a number of different proteins from several species of seeds. Most of these, which are

unquestionably the reserve proteins of these seeds, are products of the metabolism of the plant, and, in the fully ripened seed, no longer take part in the physiological processes.

Owing to their relatively great stability most of the seed proteins can be readily subjected to extensive fractional precipitation, and the chemical and physical properties of successive fractions can be easily compared. These proteins, therefore, yield more definite and well characterized preparations than do most of the well known proteins of animal origin, and it is expected that a study of them will result in more definite knowledge of the chemistry of protein matter than can be obtained by a study of proteins from any other source.

The proteins extracted from seeds are obtained in varied forms which represent distinctly different protein substances. The solubility of the protein matter in different seeds varies greatly, but in general it is found that a part is soluble in water, a part is soluble in neutral

saline solutions and a part is insoluble in either of these solutions but soluble in dilute solutions of acids and alkalies, while in the seed of the cereals a part is also soluble in alcohol of from 70 to 90 per cent.

A detailed discussion on the solubility of the vegetable proteins follows.

A. Solubility in Water.

All seeds when ground fine and treated with water, yield extracts which in most cases contain only a small quantity of the protein substance. Some of this consists of proteose and albumin, soluble in pure water; some may be globulin, dissolved in the dilute saline solutions formed by the soluble mineral constituents of the seed; and some may be protein insoluble in water alone, but dissolved by the acids extracted from the seeds.

From the soy bean, when freshly ground, water extracts a considerable quantity of the protein, which after a time begin to separate from the solution in consequence of a development of acid in the extract. The proteins that are thus

separated are soluble in neutral saline solutions.

B. Solubility in Saline Solutions.

A large proportion of the different seed proteins are soluble in neutral saline solutions from which they may be precipitated by removing the salt or by abundant dilution. Some of these proteins are but slightly soluble at room temperature in sodium chloride solutions when these contain less than 2 per cent of salt, while others are soluble in solutions containing only a few tenths of one per cent. The degree of solubility depends much on the temperature of the solution and increases rapidly at about thirty degrees.

Salts of strong bases with weak acids which are dissociated in solution with alkaline reaction have a solvent power approximately proportional to their hydrolytic dissociation. Sodium sulphite and sodium thiosulphate are alike in their solvent power, both being much better solvents than the sulphates.

Salts of weak bases with strong acids, which are hydrolytically dissociated with acid reaction,

having a less solvent power than those of strong bases with strong acids, corresponding in this respect to their hydrolytic dissociation. Thus manganese chloride, manganese sulphate and ferrous sulphate dissolve the particular protein soluble in saline solutions to about the same extent as does sodium chloride, having, therefore, about one half the solvent power of the salts of strong bases with strong acids, the two manganese salts being in fact, better solvents than the ferrous sulphate.

C. Solubility in Acids and Alkalies, and Alcohol.

A large proportion of proteins are also soluble in acids and alkalies as has been discussed under another heading. Only the proteins that are contained in the seeds of cereals are to any extent soluble in alcohol.

Many of the leguminous seeds, such as peas, beans and soy beans yield relatively much protein to water which, on adding a little acetic acid, and passing carbonic acid until the extract becomes slightly acid, is largely precipitated.

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For extraction with neutral salts the solvent employed is generally a ten per cent solution of sodium chloride. This may be used either after the ground seed has been extracted with water, in which case only those proteins soluble in the neutral saline solution are obtained in the extract or it may be applied directly to the meal, in which case the extract will also contain nearly or quite all of the proteins soluble in water. The latter method should be the one adopted. These proteins are much less soluble in cold solutions than in warm.

The amount of proteins extracted by alkalies is much greater than that extracted by neutral saline solutions or water, in fact, in many cases it is very much greater. Sufficient attention has not yet been directed to the cause of the difference, and for this reason the total protein constituents of the soy bean are not known. This difference may be due to one of several causes. The alkali may dissolve protein which in its native condition is insoluble in salt solutions

but soluble in alkali. It may dissolve proteins enclosed within unruptured cells and hence inaccessible to the action of neutral solvents. According to the older view the proteins soluble in alkalies dissolve in consequence of the formation of soluble alkaline salts, hence these proteins were designated caseins. Although protein may sometimes dissolve by alkalies, apparently this is not generally the case as the majority of the seed proteins have more pronounced basic properties than acid, and the reason that these dissolve when treated with alkalies is often due to the fact that combined acid is neutralized and the protein set free in a soluble condition.

The proteins are nitrogen compounds, containing between 15.5 to 18 per cent nitrogen. They are of high molecular weight which consist, so far as is at present known, essentially of combinations of alpha-amino acids and their derivatives. The classification and terminology of proteins recommended by a joint committee of the American Physiological Society and the Society of Biological Chemists is as follows:

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I. Simple Protein:- Protein substances which yield only alpha-amino acids or their derivatives on hydrolysis.

II. Conjugated Proteins:- Substances which contain the protein molecule united to some other molecule otherwise than as a salt.

III. Derived Proteins:- 1. Primary protein derivatives.

2. Secondary protein derivatives.

The proteins dissolved by water, saline solutions or alkalies can be precipitated by the addition of metallic salts, such as the chlorides and sulphates of magnesium and calcium. They may also be completely precipitated with ammonium sulphate or sodium sulphate at 33 degrees Centigrade. The proteins of the soy bean may be precipitated with any acid, preferably sulphuric acid or acetic acid.

Precipitation by acids may be due either to the acid uniting with a base, already combined

with the protein to form a soluble compound, or by the formation of a salt of the protein insoluble in water.

PART THREE

EXTRACTION OF THE PROTEINS

EXTRACTION OF PROTEINS FROM THE SOY BEAN.

EXTRACTION OF THE PROTEINS.

The large percentage of proteins in the soy bean, which varies from 38 to 46 per cent, is greater than in any other seed that is cultivated to the extent of this bean, and the value of its oil and meal make this bean the most logical one for the extraction of proteins to be used as a substitute for casein.

The extraction of the proteins involves five distinctly different phases. Of these five, four are largely mechanical problems, that do not seem to be especially difficult to solve. Determination of the solvent is the chemical factor of importance for extraction.

These five problems are:

I. Separation of the husks from the hulls.

II. Extraction of the oil.

III. Determination of the solvent.

IV. Separation of the protein colloid, and precipitation of the proteins.

V. Production of a moist or dry product.

EXTRACTION OF PROTEINS FROM THE SOY BEAN.

I.

Ten pounds of beans were soaked in three times their volume of water for forty-eight hours. It was necessary to prevent fermentation, which caused a very pungent odor; and sulphurous acid was added for this purpose. The beans were then dried in a gas oven, which proved unsatisfactory for two reasons; first, because of the length of time to dry the bean, and secondly because the bean became partly roasted. A small vacuum dryer was then tried. Twenty-four inches of vacuum were maintained, and heat applied by introducing exhaust steam in the jacket surrounding the dryer. The beans were dried very quickly, but were slightly roasted. The purpose of soaking the beans and then drying them was to make the separation of husks and hulls or kernels easier. The beans could be most satisfactorily dried on screens in a steam dryer.

The beans used in this investigation were about two years old and contained approximately

four per cent moisture. These beans could be cracked directly by rollers for this reason. The rollers employed were five inches in diameter and fifteen inches long. The beans were passed through these rollers four times. The final distance between rollers was one-sixteenth of an inch. At a distance less than one-sixteenth of an inch apart oil was pressed from the bean and the hulls crushed rather than being broken into finer particles. The material from the rollers was passed through a blast of air and the husks were blown away from the hulls.

II.

The hulls obtained from the above process were ground in a coffee mill. Attempts were made to express the oil in a hydraulic press with the hulls both in the coarse and fine form.

The hydraulic piston of this press is six inches in diameter, and the pressure is transmitted by a heavy oil. The plates, on which the meal was placed, are twelve inches square.

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Two pounds of coarse hulls were placed between canvas sheets and thirty tons of pressure was applied for five minutes. Very little oil was pressed out. The finely ground hulls seemed to be a little more satisfactory. When much smaller quantities of material were used more satisfactory results were obtained.

In the finely ground material the internal fiber cells are broken up and the oil is therefore more easily extracted.

Another method of the extraction of oil with the hydraulic press was tried with success. A cylindrical hole, three inches in diameter, was machined through a five inch steel cube. This cube was cut into two parts through the axis of the cylindrical hole, and bolted together by means of slots in the sides of the cube. A cylindrical plunger fitted closely into the cube, which rested on a cast iron base grooved for the drainage of the oil. The meal was subjected to a pressure of 40 tons on the six inch piston, which was

equivalent to 8000 pounds per square inch on the meal under the three inch plunger. This pressure was applied for thirty minutes.

The analysis of the hulls showed them to contain twenty per cent oil. Thirty per cent of this was extracted by hydraulic pressure. The meal cakes formed in the steel cube weighed about half a pound.

These cakes were then broken up and subjected to the solvent action of naphtha for forty-eight hours. The meal was then washed with fresh naphtha and dried in pans in a gas oven at 75 degrees Fahrenheit. This meal contained four per cent of oil. Before the extraction of any oil the meal is light orange in color. After the extraction of nearly all the oil the meal has a creamy white color.

III.

The soy bean used contains 45 per cent proteins. Extraction was attempted before the removal of any oil. The solvents used were water and sodium chloride. It would be inadvisable

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to use any alkalies due to the presence of the oil.

The meal was heated with three times its volume of water in a double boiler for thirty minutes at a temperature of 140 degrees Fahrenheit. Fifty gram samples of meal were used. The results were as follows:

Solvent.	% Protein Extracted.
Tap Water	13
10% Solution of NaCl	17.1

It was difficult to filter the solution which appears to be a colloid. The presence of the oil also hinders filtration. The liquor was siphoned off, and the proteins precipitated with tenth normal solphuric acid and separated from the solution in a centrifuge. In color the proteins and the solution from which they were precipitated was white.

Extraction was then attempted with alkali solutions on the meal which contained 4 per cent oil. In each case fifty gram samples were used

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and 400 c.c. of water. Heat was applied by injecting live steam into the alkali solution containing the meal. An average temperature of 160 degrees Fahrenheit was maintained for one hour. In all cases the percentage of the solvent used is based on the weight of the meal used. The following are the result of alkali extraction:

Per Cent Solvent.		Per Cent Proteins Extracted.
10%	Na_2HPO_4	14.0
10	Na_3PO_4	31.3
6	NaOH	30.8
5	NH_4OH	32.6
10	Na_2CO_3	26.6
10	Na_2CO_3 and 3% NaOH	38.4
15	Na_2CO_3 and 5 NaOH	40.1

The alkali solution of the proteins is yellow and of a syrupy nature.

IV.

The separation of this protein solution can be made in a centrifuge. The proteins were precipitated with sulphuric, hydrochloric and acetic acids, and the sulphates and chlorides of sodium

and magnesium. The precipitation with sulphuric acid proved to be the most satisfactory. The metallic salts with the application of heat also gave good results. More than a three per cent solution of sulphuric acid causes an immense formation of foam, probably due to the formation of carbon dioxide. 25 c.c. of three per cent sulphuric acid will precipitate all the proteins extracted from a fifty gram sample. On the addition of the sulphuric acid the yellow color disappears and the precipitate is pinkish white in color, which can be easily filtered. There is no colloid present at this point.

V.

When the above precipitate is thoroughly washed from sulphuric acid it can be put on the market in a moist state for use. It can be dried by means of a vacuum dryer, which consists of a steam jacketed outside cylinder and a concentric revolving inside drum, heated by live steam or exhaust steam.

PART FOUR

CONCLUSION

EXTRACTION OF PROTEINS FROM THE SOY BEAN.

CONCLUSION.

The investigation for a solvent for the proteins in the soy bean was in a large measure solved. There is no doubt that a combination of alkalies will be necessary for a satisfactory extraction.

In order to make the process a commercial one it would be necessary to make a maximum extraction of the oil, and the remaining meal after the extraction of the proteins could only be used as a fertilizer.

The type of machine to use for oil extraction is of great importance. If a hydraulic press is used the meal will have to be pressed hot. From available data hot pressing for one hour with a pressure of 3500 pounds per square inch yielded 35 per cent oil. The manufacturers of the Anderson expeller claim 60 per cent extraction. No data was obtainable.

The Anderson expeller is a horizontal press of heavy construction, weighing 8000 pounds. It

is driven by a six inch double belt on a twenty inch pulley running about two hundred and eighty-five revolutions per minute, and is back geared so that the main shaft or screw which forces the seed through the machine runs about fourteen revolutions per minute.

From the analysis of eighteen varieties of seeds from The Oil Miller, the Acme variety, containing 14.5 per cent oil and 45 per cent proteins, would be the logical seed to use for our purpose.

It is entirely possible to put the extraction of the proteins from the soy beans on a commercial basis.

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